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ORIGINAL RESEARCH

Assessment of Gonadotrophins among Female Thalassemic Children and Their Correlation with Serum Ferritin

[1]Dr Ganga Ram, [2]Dr Anil Kumar, [3]Dr Sanjeev Kumar Digra

[1]Senior Resident, [3]Professor, Department of Paediatrics, Government Medical College, Kathua, Jammu and Kashmir, India
[2]Lecturer, Department of Biochemistry, Government Medical College, Jammu, Jammu and Kashmir, India

Correspondence:
Dr Anil Kumar
Lecturer, Department of Biochemistry, Government Medical College, Jammu, Jammu and Kashmir, India

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ABSTRACT

Introduction: Thalassemia is an assembly of conditions which is caused from an inherited defect in the production of one or more globin chains. Endocrine defects in thalassemia major are most prevalent disturbing problems which need quick controlling and therapy.

Objective: To study the occurrence and pattern of endocrinopathies among thalassemic female children (N=55) within 6 months to 18 years of age attending thalassemia day care centre in Department of Paediatrics GMC, Jammu and to determine the correlation of gonadotrophins with serum ferritin.

Results: All of the patients (100%) had thalassemia major. Hypothyroidism and hyperthyroidism were present in 3.63% and 0.01% patients, respectively. Majority (72.72%) of the patients had lower Vitamin B12 level. Diabetes mellitus was present in only 2 patients. All patients had reduced haemoglobin level. All female patients within the age group 13-18 years had reduced LH level. Increased FSH level was present in one female patient. Serum ferritin levels were found high in all the patients. In females ≤ 10 years, megaloblastic anaemia was seen in 68.57% patients, and hypothyroidism and diabetes mellitus in 1 patient each; whereas in females >10 years, hypogonadism (LH) was noted in all patients followed by megaloblastic anaemia in 80% females, and diabetes mellitus, hypergonadism (FSH), and hypothyroidism in 1 patient each. A positive correlation was found between Serum ferritin and LH/ FSH.

Conclusion: This study shed some light on the endocrinal issues linked with thalassemia and increases the previously prevailing studies about the occurrence of endocrinopathies among thalassaemic patients. Gonadotropins were found to be correlated positively with serum ferritin level among these patients. Early detection and institution of appropriate transfusion and chelation therapy and treatment of complication are the keys to manage this disease.

INTRODUCTION

The thalassemias are a group of anaemia that result from inherited defects in the production of haemoglobin. The thalassemias are among the most common genetic disorders worldwide, occurring more frequently in the Mediterranean region **(Fawdry*et al.*, 1944).**

Thalassemias have been described in individuals of almost every descent, but the conditions are more common among certain ethnic groups. These ethnic regions are Mediterranean (including North African, and particularly Italian and Greek), Middle Eastern, African, Chinese, and Southeast Asian (including Vietnamese, Laotian, Thai, Singaporean, Filipino, Cambodian, Malaysian, Burmese, and Indonesian) **(Lokeshwar MR *et al.*,2009).**They are seen commonly in countries to which these high-frequency populations immigrate. The Maldives has the highest prevalence is 16% in people from Cyprus, 1% in Thailand, and 3-8% in populations from India, Bangladesh, China, Malaysia and Pakistan. A very low prevalence has been reported from people in Northern Europe (0.1%) Africa (0.9%) **(Galanello R *et al.*,1979).**

In India, nearly 8,000 to 10,000 new thalassaemic (Homozygous) are born every year. In Indian studies the incidence has been reported as 0.9% of all the cases of anaemia **(Marwah RK *et al.*,1994).** The beta thalassaemic gene is more commonly found in certain communities such as Punjabis (those migrated from western Pakistan), Lohanas, Sindhis, Bengalis, Guajarati's, Bhanusthalis, Khatris.

Beta thalassemia syndromes are disorders which are inherited and are characterized by deficiency in the production of beta globin chains resulting in ineffective erythropoiesis complicated by lack of affinity of circulating haemoglobin F to 2,3-diphosphoglycerate. Since reticulocytes manufacture equimolecular quantities of alpha and beta chains, mature erythrocytes contain essentially equimolecular amounts of each chain **(Hunt T *et al.*,1976).** Patients with thalassemia do not produce enough haemoglobin (Hb) A ($\alpha_2\beta_2$) because their cells cannot manufacture either the alpha or beta polypeptide chain of human haemoglobin. Alpha-thalassemia depresses only the production of the alpha chains, and beta-thalassemia depresses only the production of the beta chains. Clinically, both alpha- and beta-thalassemia may occur in the major (homozygous), intermediate, and minor (heterozygous) genetic forms and also can interact with the presence of abnormal haemoglobins in the same individual **(Marengo-Rowe AJ *et al.*,1968).** The homozygous state result in severe anaemia in infancy. When patients are homozygous for the beta thalassemia gene, they cannot make a normal beta chain (HbA). Beta ± Thalassemia indicates a mutation that makes decreased amounts of normal Beta-globin, but it is still present (HbA) **(DeBaun MR *et al.*, 2015).** As a consequence of this, repeated blood transfusions are needed to maintain life, which in turn results in excessive iron being deposited in various organs resulting in early fatalities (**Upadya SH *et al.*, 2018)**.

Endocrine dysfunction in thalassemia is amongst the most common complication and is principally attributed to excessive iron overload and suboptimal chelation. The prevalence is quite high particularly in multi-ethnic populations but determining the prevalence is often difficult due to the widespread heterogeneity of the population and timing of exposure to chelation therapy. Disturbances in growth, pubertal development, abnormal gonadal functions, impaired thyroid, parathyroid and adrenal functions, diabetes and disorderly bone growth are commonly encountered. Early detection and institution of appropriate transfusion regimen and chelation therapy and treatment of complications are the keys to managing this population including regular follow up (**Flynn DM *et al.*, 1976).** Present study was to assess the epidemiology of endocrinopathies among cases of thalassemia and to observe impacts of gonadotropins on serum ferritin level as no such study had been done in the past in our setup in already resource constrained settings.

PROCEDURE

This prospective study was conducted for one year in Hospital from 1st of November 2018 to 31st October 2019.

STUDY POPULATION

All female patients were tested for Beta thalassemia major between 6 months to 18 years of age under treatment at thalassemia day care centre, SMGS Hospital, GMC Jammu. This study was directed over a period of one year at thalassemia day care centre in the Department of Paediatrics at Govt. Medical College, Jammu from Nov 2018 to Oct 2019 after obtaining approval from Institutional Ethics Committee, Government Medical College, Jammu. A total of 55 female patients with Beta thalassemia major or intermedia between 6 months to 18 years of age and who are registered and under treatment at thalassemia day care centre, SMGS Hospital, GMC Jammu were selected for the study. Pre-structured proforma recorded a detailed history and general physical examination of patient and their peripheral blood samples were tested for complete blood count, serum ferritin, and hormonal assays like blood sugar, thyroid profile, LH, FSH.

STATISTICAL ANALYSIS

Data was entered in Microsoft Excel spreadsheet. Numbers, means, standard deviations and proportions were calculated from the data. Any seeming linkage or variances between the variables was analysed and the statistical significance was obtained using suitable statistical technique. A p- value of < 0.05 was considered as statistically significant.

RESULTS

This study was piloted over a period of one year on the female patients attending thalassemia day care centre in the Department of Paediatrics at Govt. Medical College, Jammu from Nov 2018 to Oct 2019. A total of 55 female children were used for the study. All (100 %) of them had thalassemia major. 35 of them were less than 10 years old and 20 of them were greater than 10 year of age.

The haemoglobin level was found to be less than the lower limit of the normal range (13.5-17.5) in all the patients. The mean (SD) haemoglobin was 8.39 (0.86) g/dl. The blood sugar (random) was detected within the normal range (<150) in most (96.36 %) of the patients. The mean (SD) Blood sugar (Random) was 87.72(48.80)mg/dl. This is summarised in Table 1.

Table 1: Summary of a few study points with their findings.

Study Point	Description/ Value (N=55)	Percentage/ Mean (SD)
Thalassemia Major	55	100%
Age ≤ 10 years	35	63.63%
Age > 10 years	20	36.36 %
Haemoglobin	Lower	M= 8.39 (0.86) g/dl
Blood Sugar Random	Normal	96.36%; M= 87.72 (48.80) mg/dl

Also, serum ferritin levels were greater than 204.00 ng/ml in 100% of the patients. Serum ferritin values of 4.63 -204.00 ng/ml was used as normal reference range at hospital's laboratory.

Majority of the patients (80%) had serum ferritin level greater than the 2000 (ng/ml) as presented in Table 2. The mean (SD) serum ferritin in the 55 patients was 3327.33 (1690.84) ng/ml. These levels are much greater than the normal values.

Table 2: Distribution of thalassemia patients with respect to different Serum Ferritin (ng/L) level.

Serum Ferritin (ng/ml) level.	Number of patients (N=55)
≤1000	2
1001≤2000	7
>2000	46

TSH (Thyroid Stimulating Hormone) was studied in these thalassemia patients which is summarised in Table 3. As shown in the data, majority of the patients had normal TSH level

yet 2 patients had hypothyroidism and 1patient had hyperthyroidism. Mean (SD) TSH was found to be 1.98 (1.09) µIU/ml.

Table 3: Distribution of thalassemia patients as per TSH level.

TSH level*(µIU/ml)	Number of patients (N=55)	Percentage (%)
<0.2700	1	0.01
0.2700 - 4.2000	52	94.54
>4.2000	2	3.63

* Reference value used in study Hospital laboratory.

Vitamin B12 level was tested in all these thalassemia patients. The data (Table 4) exposes that about two-third 72.72% patients had less Vitamin B12 level than even the lower limit of normal range and about one-third 27.27% of patients had Vitamin B12 within the normal range. Mean (SD) Vitamin B12 was about 159.96(37.90) pg/ml.

Table 4: Distribution of Vitamin B12 level-wise thalassemia patients.

Vitamin B12 *(pg/ml) level	Number of patients (N=55)	Percentage (%)
<187	40	72.72
187-883	15	27.27
Total	55	100

* Reference value used in study Hospital laboratory.

Table 5:- Distribution of female thalassemia patients within 13 – 18 years of age with respect to LH

LH*(U/L)	Number of patients (N=20)	Percentage (%)
<7.59	20	100
(7.59-89.08)	0	0
Total	20	100

* Reference value used in study Hospital laboratory.

Luteinizing Hormone (LH) was assessed in female patients within age group of 13 to 18 years. As per the obtained results, all the patients (100%) within the specified age group had less LH than the lower limit of the normal range as mentioned in Table 5. Mean (SD) LH was found to be 5.34(1.57)U/L. We also calculated correlation coefficient (R) between the ferritin and LH level in the above patients. The R value between the two variables was found to be 0.29, suggesting a weak positive correlation between Ferritin level and the LH level in the patients.

Similarly, follicle stimulating hormone (FSH) was also assessed in these patients and the data is summarised in Table 6. Approximately all the female patients (95%) within the age group (13-18) years had FSH level within the normal range, only one patient (5%) have raised FSH level. The mean (SD) FSH was found to be 13.08(2.45)U/L. We also calculated correlation coefficient (R) between the ferritin and FSH level in the above patients. The R value between the two variables was found to be 0.52, suggesting a good positive correlation between Ferritin level and the FSH level in the patients.

Table 6: Distribution of female thalassemia patients with respect to FSH

FSH*(U/L)	Number of patients (N=20)	Percentage (%)
(2.55-16.60)	19	95
>16.60	1	5
Total	20	100

* Reference value used in study Hospital laboratory.

The distribution of the endocrinopathies based on the above data is presented in Table 7. The data is divided in two age groups which are less than equal to 10 and greater than 10 years. We evaluated endocrinopathies like hypothyroidism, megaloblastic anaemia, diabetes mellitus and hypogonadism. Majority of patients (68.57%) had Megaloblastic-anaemia in the

age group (≤ 10 years). In the age group (> 10 years) patients had 100 % hypogonadism and 80% Megaloblastic-anaemia. Overall 2 patients (3.64%) had hypothyroidism and diabetes mellitus and 1 patient (5%) in the age group 13 to 18 years had hypergonadism.
Prevalence of endocrinopathies based on the parameter like Ferritin, TSH, Vitamin B12, random blood sugar is defined in Table 8.

Table 7: Distribution of endocrinopathies at different age groups in patients

Endocrinopathies	Number of patients N=35(%) (≤ 10 years)	Number of patients N=20(%) (> 10 years)	Total Number of patients N=55(%)
Hypothyroidism	1(2.86)	1(5.00)	2(3.64)
Megaloblastic anaemia	24(68.57)	16(80.00)	40(72.72)
Diabetes Mellitus	1(2.86)	1(5.00)	2(3.64)
Hypogonadism (L H)		20(100%)	20(100%)
Hypergonadism (FSH)		1(5.00%)	1(5.00%)

Table 8: Prevalence of endocrinopathies in thalassemia patients.

Parameter	Prevalence %
Serum Ferritin (>204)	100
TSH (<0.27)	0.01
TSH (>4.20)	3.63
Vitamin B12 (<187)	72.72
Blood Sugar (R) (>150)	3.64
LH(<7.59)	100(83.89-100)
FSH(>16.60)	5(0.89-23.60)

DISCUSSION

Thalassemia refers to a group of genetic disorders of globin chain production in which there is an imbalance between the alpha globin and beta globin chain production **(DeBaun MR *et al.*, 2015).** Patients with thalassemia do not produce enough haemoglobin A because their cells cannot manufacture either the alpha or beta polypeptide chain of human haemoglobin.
In our study, all the patients were having serum ferritin level greater than the upper limit of the normal range. This might be due to the iron overload by chelation therapy as almost all children were suffering from thalassemia major. **Soliman AT *et al.*, (2013a)** observed that the mean serum ferritin (2758 ug/L) denoted improper iron chelation in thalassemia patients. These findings suggest that thalassemia major patients predominantly develop higher iron stores subsequent to chelation therapy. The precise underlying mechanism(s) of iron-induced organ dysfunction is not much clear.
In Beta Thalassemia Major (BTM) patients the frequency of hypothyroidism ranges from 6 to 30 % among various countries depending on chelation strategies. The quantity and duration of iron overload mainly determine the prognosis among such patients. Iron deposition from repeated transfusions has been implicated as the likely mechanism causing thyroid dysfunction in BTM patients. In our study, 95.54% patients had normal TSH level following 3.63% approximately had hypothyroidism and 0.01% approximately had hyperthyroidism respectively. Among the other studies, **SolimanAT *et al.*, (2013a)** found that the hypothyroidism was diagnosed in 35% of patients, which was quite high as compared to our study. In study by **Upadya SH *et al.*, (2018)** 4.8% of the children had evidence of subclinical hypothyroidism.

Thalassemic patients are at risk of micronutrient deficiency. They have ineffective erythropoiesis and accelerated red cell turnover owing to the short life span of RBC which results in increased body demand of energy and nutrients to maintain normal erythropoiesis. Vitamins and trace minerals represent key buffers against oxidative damage. (**Sherief LM *et al.*, 2014)**. In our study, 72.72% patients had Vitamin B12 level less than the lower limit of normal range. **Sherief LM *et al.*, (2014)** reported that there was a significant decrease of vitamin B12 in thalassemic patients as compared to controls. These findings show that patients with B-thalassemia-major have significant deficiencies of various nutritional markers which could be attributed to inadequate intake in the face of increased demand, consumption, and excretion.

In patients with β-thalassemia major (BTM), hyper-transfusion therapy has dramatically increased the duration and quality of life but has been associated with chronic iron overload, and frequently complicated by the development of diabetes mellitus (DM) or impaired glucose tolerance (IGT). DM is still responsible for significant morbidity and mortality in thalassemic patients. Prevalence has been reported to range from 2.3% to 24% (**SolimanAT*et al.*, 2013b)**. In our study, most of the patients had Blood sugar random within the normal range except 2 patients. **Soesanti*et al.*, (2013)** found thatnone of the patients was diagnosed as DM. **Gamberini MR *et al.*, (2008)** observed diabetes mellitus (DM) in 17% of patients.

Ineffective bone marrow erythropoiesis and excessive red blood cell haemolysis together account for the anaemia. The anaemia is due to a combination of ineffective erythropoiesis, excessive peripheral red blood cell haemolysis, and progressive splenomegaly **(Marengo-Rowe AJ, 2007)**. Thalassemia major is characterized by reduced Hb level (<7 g/dl), mean corpuscular volume (MCV) > 50 < 70 fl and mean corpuscular Hb (MCH) > 12< 20 pg. Thalassemia intermedia is characterized by Hb level between 7 and 10 g/dl, MCV between 50 and 80 fl and MCH between 16 and 24 pg. Thalassemia minor is characterized by reduced MCV and MCH, with increased Hb A^2 level **(Galanello R *et al.*, 2010)**. In our study, all the patients have haemoglobin level less than the lower limit of the normal range. Similar to our study, **Sherief LM *et al.*, (2014)** also reported lower level of haemoglobin in children with thalassemia. Mean Hb level was 7.39 g/dL in their study.

Hypogonadism is the most frequently reported endocrine complication, affecting 70–80% of thalassemia major patients. Hypogonadism is likely to be caused by iron deposits in the gonads, pituitary gland or both. This can be assessed by checking LH and FSH levels in female patients above 13 years of age. In our study, mean FSH was 13.08 U/L. Most of the female patients (95%) within the age group (13-18) years had FSH level within the normal range, only one patient (5%) have raised FSH level. **Haggag AA *et al.*, (2016b)** found that mean FSH level was 1.17 mIU/ml in females with thalassemia with iron overload and that in thalassaemic patients without iron overload was 2.55 mIU/ml.

In our study, mean value of LH was 5.34 U/L. All the female patients within the age group (13-18) years had LH level less than the lower limit of the normal range. Our finding was similar to that of **Haggag AA *et al.*, (2016b)** who reported that mean LH level was 0.98 mIU/ml in females with thalassemia with iron overload and that in thalassaemic patients without iron overload was 1.91 mIU /ml. We also calculated correlation coefficient (R) between the ferritin and LH/ FSH level in the above patients. The R value between the two variables was found to be 0.29 and 0.52 for LH and FSH respectively, suggesting a positive correlation between Serum Ferritin and the LH/ FSH level in the patients. This was in contrast with the data obtained by **Ansaaf et al., 2021.**

CONCLUSION

In present study, all of the patients (100%) were having major type of thalassemia. Hypothyroidism and hyperthyroidism were present in 3.63% and 0.01% patients,

respectively. Majority (72.72%) patients had Vitamin B12 level less than the normal range. Diabetes mellitus was present in only two patients. All patients had reduced haemoglobin level. All female patients within the age group 13-18 years had reduced LH level. Increased FSH level was present in one female patient. In females ≤ 10 years, megaloblastic anaemia was seen in 68.57% patients, and hypothyroidism and diabetes mellitus in 1 patient each; whereas in females >10 years, hypogonadism (LH) was noted in all patients followed by megaloblastic anaemia in 80% females, and diabetes mellitus, hypergonadism (FSH), and hypothyroidism in 1 patient each. A positive correlation was found between Serum ferritin and LH/ FSH.

The combination of early diagnosis, improvement in monitoring complications and advanced supportive therapy has enabled patient with thalassemia major to have improved life expectancy **(Britton RS, Rammga*et al.*, 1994).** The cornerstone in the management is life-long blood transfusion with frequent iron chelation therapy to minimize the deleterious effects of chronic iron deposition and accumulation of tissue. Despite this, these patients are prone to long term organ dysfunction particularly the cardiovascular, hepato- biliary, endocrine and skeletal system (**Thein SL, Clegg JB *et al.*, 1998).** Chronic blood transfusion therapy dramatically improves the quality of life of thalassemic patients and also reduces the complications of thalassemia. Early detection and institution of appropriate transfusion and chelation therapy and treatment of complication are the keys to manage this population including regular follow up (**Flynn DM *et al.*, 1976).** The prevalence quite high particularly in multi-ethnic population what determine the prevalence is often difficult due to wide spread heterogeneity of the population and timing of exposure to chelation therapy (**Flynn DM *et al.*, 1976).**

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